PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 31 May 2001 (31.05.01)	
International application No. PCT/AU00/01193	**Applicant's or agent's file reference** 626091
International filing date (day/month/year) 29 September 2000 (29.09.00)	**Priority date** (day month year) 29 September 1999 (29.09.99)
Applicant EDGAR, John, Alexander	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

26 April 2001 (26.04.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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Facsimile No.: (41-22) 740.14.35

Authorized officer

J. Leitao

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01193

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl A61K 38/12, A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 38/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, CAPLUS, MEDLINE, keywords, phomopsin, cancer, tumor.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN ASWEGEN, C. H. et al "INFLUENCE OF PHOMOPSIN AND IVALIN ON STEROID-HORMONE BINDING AND GROWTH OF MCF-7 HUMAN BREAST CANCER CELLS." Journal of Toxicology and Environmental Health, VOL 16(1), 1985 pages 13-23. See whole document.	1-19
X	LI, YIN et al "INTERACTION OF PHOMOPSIN A WITH PORCINE BRAIN TUBULIN " Biochemical Pharmacology, Vol 43, No 2, pages 219-224, 1992. See whole document.	17-19

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents.
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
19 October 2000

Date of mailing of the international search report
1 NOV 2000

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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01193

C (Continuation). **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKAHASHI, M et al "SYNTHETIC STUDY OF USTILOXIN ANALOGS. BENZYLIC OXIDATION OF 13-MEMBERED CYCLIC PEPTIDE BY LEAD TETRAACETATE." HETEROCYCLES, Vol 47, No 1, 1998 pages 163-166. See Whole document	17-19
X	AU 64916/90 (643464)B (CSIRO) 23 October 1990. See Whole document.	17-19

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference IRN626091	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU00/01193**	International Filing Date *(day/month/year)* 29 September 2000	Priority Date *(day/month/year)* 29 September 1999
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] A61K 38/12, A61P 35/00		
Applicant COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
26 April 2001	1 August 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **G.R.PETERS** Telephone No. (02) 6283 2184

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU00/01193

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
[redacted]/AU00/01193

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-16**	**YES**
	Claims	**17-19**	**NO**
Inventive step (IS)	Claims		**YES**
	Claims	**1-19**	**NO**
Industrial applicability (IA)	Claims	**1-19**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

Novelty (N) and Inventive Step (IS) claims 1-19.

Citations

1. VAN ASWEGEN, C. H. et al "INFLUENCE OF PHOMOPSIN AND IVALIN ON STEROID-HORMONE BINDING AND GROWTH OF MCF-7 HUMAN BREAST CANCER CELLS." Journal of Toxicology and Environmental Health, Vol 16(1), 1985 pages 13-23.

2. LI, YIN et al "INTERACTION OF PHOMOPSIN A WITH PORCINE BRAIN TUBULIN." Biochemical Pharmacology, Vol 43, No 2, 1992 pages 219-224,.

3. TAKAHASHI, M et al "SYNTHETIC STUDY OF USTILOXIN ANALOGS: BENZYLIC OXIDATION OF 13-MEMBERED CYCLIC PEPTIDE BY LEAD TETRAACETATE." Heterocycles, Vol.47, No 1.1998 pages 163-166.

4. AU 64916/90 (643464)B.

Citation 1 discloses the dose-dependent inhibition of breast cancer cells in culture (page 22) using a phomopsin composition. Claims 1-16 lack an inventive step in the light of this document as it would have been obvious for a person skilled in the art to at least try the phomopsin compositions of the citation (the same compositions as used in present claims 1-16) in the treatment of a patient afflicted with cancer.

Claims 17-19 define pharmaceutical compositions comprising a phomopsin. The compositions are not restricted to the use stated in the claims and as such the novelty of claims 17-19 is destroyed by each of the four listed citations as they all describe compositions comprising a phomopsin.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/22986 A1

(51) International Patent Classification[7]: A61K 38/12, A61P 35/00

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29 September 2000 (29.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 3148 29 September 1999 (29.09.1999) AU

(71) Applicant *(for all designated States except US)*: **COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION** [AU/AU]; Limestone Avenue, Campbell, ACT 2612 (AU).

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(75) Inventor/Applicant *(for US only)*: **EDGAR, John, Alexander** [AU/AU]; 1/173 Ormond Road, Elwood, VIC 3184 (AU).

(74) Agent: **PHILLIPS ORMONDE & FITZPATRICK**; 367 Collins Street, Melbourne, VIC 3000 (AU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
With international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/22986 A1

(54) Title: ANTI CANCER AGENT AND METHOD OF TREATMENT OF CANCER

(57) Abstract: A method of treatment of a patient suffering cancer comprising administering to the patient an effective amount of a phomopsin.

ANTI CANCER AGENT AND METHOD OF TREATMENT OF CANCER

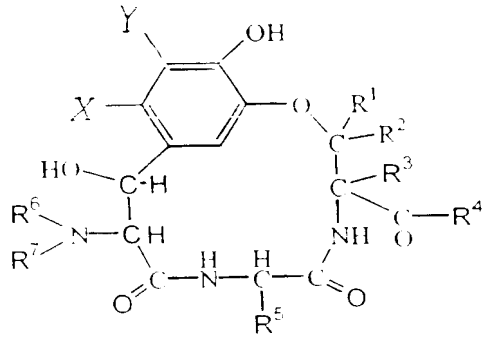
The present invention relates to the treatment of cancer and to compositions for use in treatment of cancer.

The search for anti-cancer agents has been, and remains, a major endeavour of the pharmaceutical industry, academic institutions and government agencies throughout the world. One of the significant problems with many cancer treatments is the severe adverse affects they have on the patient and non-cancerous tissues.

We have now found that phomopsin mycotoxins (hereafter referred to as phomopsins) and their derivatives exhibit potent anticancer activity. In addition, and due to the tendency of phomopsins to specifically target the liver, we believe that phomopsins may be used to provide selective activity against liver cancer. It will be appreciated that the selectivity of phomopsins in treatment of liver cancer is a significant advantage as it allows liver cancers to be targeted while minimising the effects on other tissues.

Phomopsins may however be utilised in treatment of cancers other than liver cancer by selecting formulations or derivatives of phomopsins which enhance selectivity of the drug for certain types of cancer cells or certain types of cancers. Derivatives of phomopsins may be formed which are conjugates with monoclonal antibodies. The monoclonal antibody may be produced by known methods to provide selectivity for cancer cells.

Phomopsins are characterised by a 13-member ring structure generally of formula I



(I)

wherein

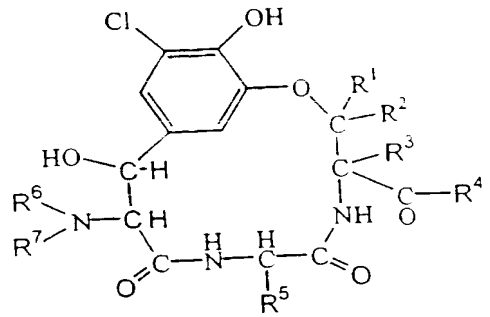
R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are optional substituents and may be independently selected from the group consisting of hydrogen, aliphatic, aromatic, peptide chains and halogen.

X is aliphatic, hydrogen or halogen (preferably hydrogen); and

Y is aliphatic, hydrogen or halogen (preferably chlorine);

where present a peptide chain may be conjugated with a monoclonal antibody (Mab). The phomopsins may be derivatives of compounds of formula I such as the salts thereof.

The preferred phomopsins as selected from compounds containing the group of formula Ia:

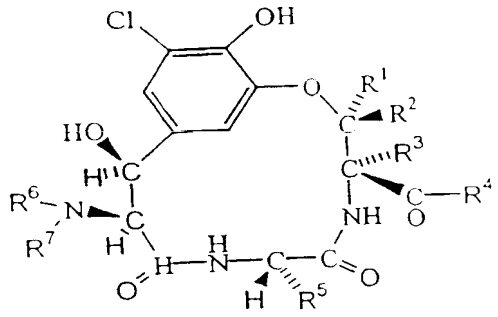


(Ia)

and the derivatives thereof.

In formula I and Ia R^1, R^2, R^3, R^5, R^6 and R^7 may typically be independently selected from hydrogen and aliphatic and R^4 is generally a peptide. In one embodiment R^4 is a peptide conjugated with an antibody, particularly a monoclonal antibody (Mab). More preferably R^1, R^2, R^5 and R^6 are lower aliphatic and R^3 and R^7 are hydrogen. Even more preferably R^1, R^2 and R^6 are lower alkyl and R^5 is lower alkyl or lower alkenyl. Most preferably R^1 is ethyl, R^2 is methyl, R^3 is hydrogen, R^5 is isopropyl or iso-propenyl and R^6 is methyl. Where used herein the terms lower aliphatic, lower alkyl and, lower alkenyl include groups containing up to six carbon atoms and most preferably up to 4 carbon atoms.

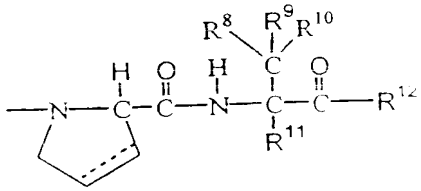
The preferred stereochemistry of the compounds of formula Ia is as shown in formula Ib:



(Ib)

Preferably at least 60% by weight of the phomopsin component will have stereochemistry 1b.

The group R^4 is a peptide preferably a di- or tri-peptide which may optionally be bound to an antibody such as a monoclonal antibody. The preferred group R^4 has the formula II and includes all stereo isomers:

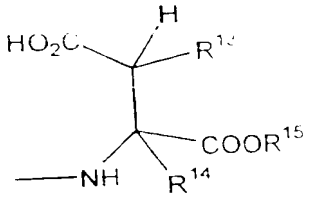


(II)

wherein the dotted line represents an optional double bond;

R^8 and R^9 are independently selected from hydrogen and lower alkyl and more preferably R^8 is methyl and R^9 is ethyl and R^{11} and R^{10} are hydrogen or together form a double bond;

R^{12} is selected from the group consisting of amino, mono substituted amino, disubstituted amino and an amino acid residue particularly the group of formula III.

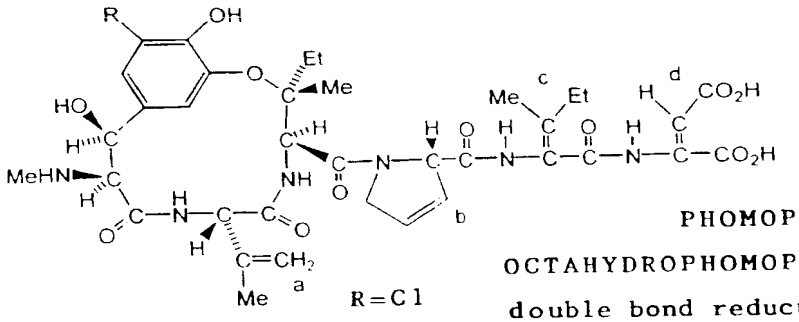


(III)

wherein R^{13} and R^{14} are hydrogen or together form a double bond and R^{15} is selected from the group consisting of hydroxy, amino, substituted amino or an antibody particularly Mab.

When R^{15} is an antibody or linked to an antibody it is preferred that R^{13} and R^{14} form a double bond providing a dehydroaspartic acid residue. In such a case, the carbon-nitrogen bond in the residue of formula III is relatively weak enabling an active phomopsin of formula Ia (wherein in the group of formula II R^{12} is amino) to be released from the MAb once it becomes bound to cancer cells. Thus a dehydroaspartic acid residue is expected to facilitate delivery of phomopsins via the Mab conjugate.

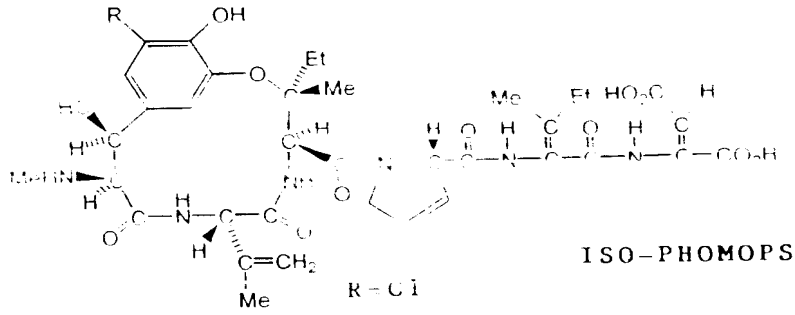
The most preferred phomopsin compounds are selected from phomopsin A, octahydrophomopsin A, iso-phomopsin A and phomopsinamine A. These compounds have the formula set out below:



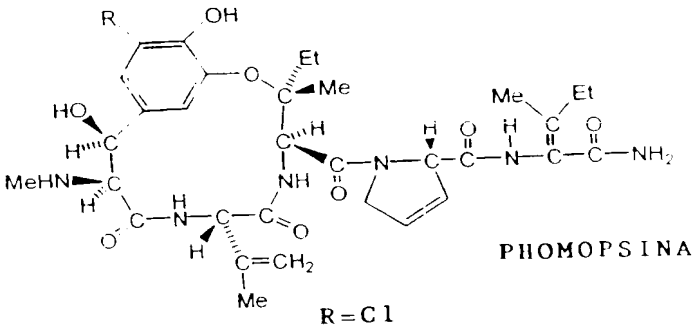
PHOMOPSIN A MW 788

OCTAHYDROPHOMOPSIN A MW 796

double bond reduction sites a, b, c, d



ISO-PHOMOPSIN A MW 788



PHOMOPSINAMINE A MW 674

R=Cl

The patent may be treated with a mixture of phomopsins and it will be understood that the reference to phomopsin in the specification and claims includes mixtures of phomopsins.

In one aspect the invention provides a pharmaceutical composition for treatment of cancer, preferably liver cancer, containing a phomopsin compound or derivative thereof or pharmaceutically acceptable salt of the phomopsins or derivative and a pharmaceutically acceptable carrier.

Salts of phomopsins such as the alkaline metal salts are reasonably water soluble. Aqueous solutions can be formed by dissolving the phomopsins in a dilute base such as sodium hydroxide to provide a neutral solution.

In another aspect the invention provides a method of treatment of a patient suffering cancer including administering to the patient a phomopsin compound or derivative thereof or pharmaceutically acceptable salt of the phomopsin or derivative.

The phomopsin compound may be administered by a variety of methods including oral administration in the form of a syrup capsule tablet or the like by injection or by intravenous infusion

Preferably the compound is administered by intravenous infusion

In a further aspect the invention provides the use of a phomopsin compound as hereinbefore described for preparation of a pharmaceutical composition for treatment of cancer and in particular liver cancer

Phomopsin compounds are produced by certain fungi, including *Diaporte toxicus* (formerly *Phomopsis leptostromiformis*) and *Phomopsis emicis*, or may be derived from these natural products.

The activity of phomopsins is believed to be due in part to the strong binding of the compounds to tubulin. This may disrupt cell mitosis by inhibiting tubulin formation and cause depolymerization of formed microtubules. It may be preferred in some cases to use phomopsins in combination therapy with one or more other anticancer drugs or therapies. The drugs used in combination with phomopsins may be selected to enhance results by providing complementary activity in binding to microtubules. Examples of possible drugs for use in combination with phomopsins include paclitaxel, vinblastine and vincristine.

The invention will now be described with reference to the following examples. It is to be understood that the examples are provided by way of illustration of the invention and that they are in no way limiting to the scope of the invention.

Examples

For the *in vitro* and *in vivo* assessments of anticancer activity performed by the National Cancer Institute in the USA, phomopsin A, iso-phomopsin A, phomopsinamine A and octahydrophomopsin A were obtained by the methods as described in the references by C. Culvenor, J. Edgar and M. Mackay, Tetrahedron Vol. 45, No. 8 pp 2351 (1989). and by J. Edgar, J. Frahn, P. Cockrum and J. Culvenor in the paper "Lupinosis. The Chemistry and Biochemistry of the Phomopsins" Mycotoxins and Phycotoxins, collection of invited papers presented at the sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Rep. South Africa, 22-25 July 1985, or as described herein.

ISOLATION OF PHOMOPSIN A

Background:

The extraction process is designed to minimise difficulty and cost. The fermented seed is continuously extracted with recycling 15% methanol:water

through an in line XAD (styrene divinylbenzene copolymer) column. The time required for adsorption of phomopsin A onto the XAD is quite lengthy, but requires minimal operator input. The timing of this step is not critical, hence can be adapted to suit operating conditions.

The phomopsin A has a relatively low solubility in 15% methanol. The procedure relies on the adsorption of phomopsin A on the XAD resin driving the solubility equilibrium of phomopsin A in the fermented seed toward dissolution. This procedure reduces solvent usage, volumes to be handled and flammability hazards. The alternate method of extraction, without recycling would use 150+ litres of pure methanol for the initial extraction, involve a further concentration step (or dilution of the methanol extract to 900+L) then adsorption onto XAD. The current procedure uses 12 L methanol, requires minimal operation input for the adsorption phase and uses far less solvent (total volume 85L instead of 900+L).

The elution of the concentrated phomopsin A from the column is the first step in a 3 stage isolation to produce crystalline phomopsin A of 80-90% purity.

After a preliminary wash with 15% methanol in water, phomopsin A may be eluted from the column using 100% methanol. Silica gel flash column chromatography may be used for purification. The column is conditioned using 5:95 ammonia:isopropanol and the concentrate dissolved in a minimum of 20:65:15 ammonia:isopropanol:water. Phomopsin A is eluted using this 3 solvent combination. Recrystallisation from boiling glacial acetic acid provides phomopsin A in 80-90% purity.

PREPARATION OF *iso*-PHOMOPSIN A

Materials:

0.5M $HgCl_2$: 280 mg $HgCl_2$ dissolved in 2 ml H_2O (+50 µl 10M HCl)

0.01M Phomopsin A: 18.3 mg PhA dissolved in 2 ml H_2O (with puff of NH_3).

1M HCl

Method:

0.01M Phomopsin A (2.0 ml) was mixed with 0.5M $HgCl_2$ (1 ml) and 1M HCl (200 µl), total volume 3.2 ml, and left at room temperature for 5 hours. The solution was diluted to 8 ml with water then passed through a prepared C18 Maxi-clean SPE cartridge (900 mg) and washed with 7-8 ml H_2O. The adsorbed *iso*-phomopsin A was then eluted with 8-9 ml MeOH The aqueous eluate from the first C18 cartridge was reprocessed through a second C18 cartridge to check whether the first cartridge was overloaded. The MeOH eluate from the second cartridge had very little residue on drying and was not included in further processing.

The methanol eluate, made up to 10mls, was analysed by HPLC and then was evaporated to dryness and purified using preparative HPLC.

PREPARATION OF PHOMOPSINAMINE A

Phomopsin A (15.3 mg) was dissolved in the minimum amount of 1M HCl and left at room temperature for 28 hours. The reaction mixture was diluted to 8 ml with water then passed through a strong anion exchange cartridge (SAX, 600 mg) to remove any unreacted phomopsin A (pH of solution expected to be about 1.52). The aqueous solution of non-adsorbed compounds, and the water washings of the SAX column, were then passed through a prepared C18 cartridge (900 mg). The C18 cartridge was washed with H_2O (10 ml) then the phomopsinamine A eluted with methanol (10 ml).

The methanol eluate was subjected to HPLC analysis and then evaporated to dryness and the phomopsinamine A purified using preparative HPLC.

This method may be modified by sampling the reaction mixture after 5-6 hours 24 hours and 28-30 hours. All washings and eluates may be assayed by HPLC to monitor the conversion of phomopsin A to phomopsinamine A

ANTICANCER ACTIVITY OF THE PHOMOPSINS

In vitro Screening Assay

The anticancer activity of phomopsin A, octahydrophomopsin A, iso-phomopsin A and phomopsinamine A was assessed against 60 human cancer cell lines *in*

vitro. The methods used to assess anticancer activity are those employed by the United States National Cancer Institute (NCI) as a primary screen for discovering compounds with anticancer potential (Boyd and Paull, Drug Development Research, 34, 91-109 1995).

The measured effect of the compound on the Percentage Growth (PG) of a cell line is currently calculated according to one or the other of the following two expressions:

If (Mean OD_{test} - Mean OD_{tzero}) $\geq$ 0, then

$$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } OD_{tzero})/(\text{Mean } OD_{ctrl} - \text{Mean } OD_{tzero})$$

If (Mean OD_{test} - Mean OD_{tzero}) $<$ 0, then

$$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } OD_{tzero})/\text{Mean } OD_{tzero}$$

Where:

Mean OD_{tzero} = The average of optical density measurements of SRB-derived color just before exposure of cells to the test compound.

Mean OD_{test} = The average of optical density measurements of SRB-derived color after 48 hours exposure of cells to the test compound.

Mean OD_{ctrl} = The average of optical density measurements of SRB-derived color after 48 hours with no exposure of cells to the test compound.

Results

The calculated PGs of each of 60 cell lines for various concentrations of the test compounds are presented in Tables 1a to 4b. Testing was conducted twice for each compound and the results of the testing of this compound phomopsin A (Tables 1a and 1b), octahydrophomopsin A (Tables 2a and 2b), isophomopsin A (Tables 3a and 3b) and phomopsinamine A (Tables 4a and 4b) and demonstrate a dose-related response of most of the cancer cell lines tested to phomopsin A, iso-phomopsin A, octahydrophomopsin A and phomopsinamine A. In particular, the data supported progression of the assessment procedure to *in vivo* testing.

Table 1a

Compound 1 Phomopsin A
ID No 9502RM16

	Log10 Concentration				
	Percent Growth				
Cell line	-6	-7	-6	-5	-4
Leukemia					
CCRF-CEM	99	106	98	34	-24
HL-60 (TB)	101	101	76	17	-43
K-562	97	102	87	24	-23
MOLT-4	99	103	95	43	29
RPMI-8226	111	109	103	36	-5
SR	118	111	53	-16	-35
Non-small cell lung cancer					
A549/ATCC	102	103	69	31	17
EKVX	102	106	85	40	11
HOP-62	104	106	96	72	56
HOP-92	114	116	110	91	90
NCI-H226	107	121	67	-4	-26
NCI-H23	105	102	101	74	36
NCI-H322M	102	98	94	30	8
NCI-H460	103	111	83	16	9
NCI-H522	103	105	100	18	-21
Colon cancer					
COLO 205	107	75	57	-54	-79
HCC-2998	95	95	74	4	-46
HCT-116	97	102	101	32	11
HCT-15	90	97	74	26	10
HT29	95	97	91	14	5
KM12	100	83	62	12	5
SW-620	96	107	101	62	44
CNS cancer					
SF-268	101	101	89	51	36
SF-295	107	102	74	21	8
SF-539	94	94	69	-18	-54
SNB-19	93	97	92	44	22
SNB-75	93	77	48	-11	21
U251	100	102	89	16	4
Melanoma					
LOX IMVI	90	97	82	43	20
MALME-3M	101	92	65	32	25
M14	98	83	64	0	-27
SK-MEL-2	97	95	84	32	11
SK-MEL28	94	83	68	44	37
SK-MEL-5	100	87	48	30	23
UACC-257	113	104	79	56	70
UACC-62	101	95	79	38	26
Ovarian cancer					
IGR-OV1	99	102	97	73	44
OVCAR-3	102	97	64	11	4
OVCAR-4	99	84	114	63	54
OVCAR-5	101	102	78	20	27
OVCAR-8	95	99	97	61	8
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Renal cancer					
786-0	105	96	86	29	18
A498	94	[illegible]	[illegible]	[illegible]	[illegible]
ACHN	101	91	83	45	30
CAKI-1	90	88	80	37	28
RXF-393	91	87	49	25	39
SN12C	104	104	92	64	38
TK-10	94	101	93	68	53
UO-31	98	94	91	54	48
Prostate cancer					
PC-3	100	89	66	20	10
DU-145	108	102	66	9	-13
Breast cancer					
MCF7	98	92	67	21	5
MCF7/ADR-RES	99	99	90	48	13
MDA-MB-231/ATCC	99	101	95	77	2
HS 578T	104	107	101	71	75
MDA-MB-435	98	60	1	4	80
MDA-N	93	71	29	6	-29
BT-549	100	121	11	1	54
T-47D	91	107	71	41	72

Table 1b

Compound 1 Phomopsin A
ID No: 9409SC89

	Log10 Concentration Percent Growth				
Cell line	-6	-7	-6	-5	-4
Leukemia					
CCRF-CEM	84	86	80	-2	-47
HL-60 (TB)	75	88	76	-31	-65
K-562	105	121	93	33	11
MOLT-4	98	93	90	28	28
RPMI-8226	103	94	87	9	-27
SR	90	88	88	25	19
Non-small cell lung cancer					
A549/ATCC	107	103	82	34	25
EKVX	107	99	92	58	45
HOP-62	100	114	99	57	35
NCI-H226	87	85	96	34	-5
NCI-H23	101	101	88	20	-2
NCI-322M	93	93	80	27	44
NCI-H460	101	95	80	12	
NCI-H522	102	102	93	9	-21
Colon cancer					
COLO 205	99	108	69	-27	-44
HCC-2998	104	96	87	11	-37
HCT-116	102	94	93	32	15
HCT-15	102	99	103	35	15
HT29	95	95	92	-14	-5
KM12	92	87	61	-19	-52
SW-620	104	104	93	34	22
CNS cancer					
SF-268	104	106	87	40	16
SF-295	100	94	74	-45	-53
SF-539	99	102	98	32	-7
SNB-19	101	98	94	56	33
SNB-75	83	55	28	15	15
U251	103	98	91	26	9
Melanoma					
LOX IMVI	101	108	100	46	37
M14	99	110	76	19	-31
SK-MEL-2	87	92	74	32	16
SK-MEL-28	96	98	69	37	51
SK-MEL-5	106	101	54	22	10
UACC-257	98	92	75	26	42
Ovarian cancer					
IGROV1	104	119	109	57	27
OVCAR-5	99	100	82	24	19
OVCAR-8	105	133	104	59	30
SK-OV-3	94	114	89	26	47
Renal cancer					
786-0	91	97	98	38	17
A498	99	100	83	17	-5
ACHN	93	93	90	41	1
SN12C	105	100	103	59	28
TK-10	100	106	98	99	55
Prostate cancer					
PC-3	99	100	86	21	1
DU-145	99	105	83	22	7
Breast cancer					
MCF7	98	100	76	23	12
MCF7/ADR-RES	97	100	99		
MDA-MB-231/ATCC	99	98	96	66	34
HS 578T	113	109	79	23	-4
MDA-MB-435	90	81	34	1	-23
MDA-N	103	101	23	-75	-83
BT-549	107	110	100	65	43
T-47D	95	98	74	32	57

Table 2a

Compound 1 Octahydrophomopsin A
ID No 9409SC89

	Log10 Concentration Percent Growth				
Cell line	-6	-7	-6	-5	-4
Leukemia					
CCRF-CEM	90	96	82	8	-3
HL-60 (TB)	106	95	88	-25	-49
K-562	100	108	92	22	14
MOLT-4	103	112	105	39	20
RPMI-8226	110	99	84	7	-33
SR	94	96	92	27	15
Non-small cell lung cancer					
A549/ATCC	105	104	97	47	12
EKVX	95	97	88	62	44
HOP-62	103	96	105	72	43
NCI-H226	85	75	85	33	-22
NCI-H23	110	118	104	69	12
NCI-H322M	100	99	91	49	26
NCIH460	99	99	96	29	2
NCIH522	99	99	88		5
Colon cancer					
COLO 205	97	100	70	1	-82
HCC-2998			135		-16
HCT-116	104	103	100	41	14
HCT-15	96	97	97	58	16
HT29	93	91	90	30	6
KM12	108	124	139	63	-8
SW-620	95	98	87	54	32
CNS cancer					
SF-268	101	100	86	43	22
SF-295	88	88	72	-27	-65
SF-539	101	95	95	27	-32
SNB-19	100	97	99	59	38
SNB-75	90	111	89	7	27
U251	96	99	90	20	-3
Melanoma					
LOX IMVI	97	100	92	52	39
M14	101	70	94	24	-51
SK-MEL-2	111	109	106	38	60
SK-MEL-28	105	94	69	29	41
SK-MEL-5	93	105	45	3	-19
UACC-257	98	97	85	36	37
Ovarian cancer					
IGROV1	108	107	99	52	34
OVCAR-5	102	94	96	57	24
OVCAR-8	106	99	100	62	29
SK-OV-3	85	98	79	27	-11
Renal cancer					
786-0	94	111	91	41	4
A498	104	100	103	49	16
ACHN	99	96	89	61	22
SN12C	96	96	94	47	8
TK-10	97	96	101	67	41
Prostate cancer					
PC-3	89	100	94	47	21
DU-145	105	108	95	29	-4
Breast cancer					
MCF7	115	108	108	34	26
MCF7/ADR-RES	107	106	105	66	-15
MDA-MB-231/ATCC	100	95	83	53	23
HS 578T	90	90	72	13	-10
MDA-MB-435	100	91	59	13	-14
MDA-N	101	99	51	-9	-23
BT-549	111	75	87	57	37
T-47D	99	122	89	45	48

Table 2b

Compound 1 Octahydrophomopsin A
ID No: 950RM16

	Log10 Concentration Percent Growth				
Cell line	-6	-7	-6	-5	-4
Leukaemia					
CCRF-CEM	99	102	93	28	-33
HL-60 (TB)	100	81	93	2	-33
K-S62	104	100	99	27	-21
MOLT-4	104	99	103	46	26
RPMI-8226	94	87	96	39	-3
SR	78	92	54	-9	-35
Non-small cell lung cancer					
A549/ATCC	104	93	101	57	21
EKVX	105	93	93	52	23
HOP-62	89	90	85	55	21
HOP92	98	99	92	51	76
NCI-H226	108	104	102	25	-14
NCI-H23	94	96	85	57	27
NCI-H322M	99	98	99	74	17
NCI-H460	105	101	104	45	15
NCI-H522	108	103	102	27	-33
Colon cancer					
COLO 205	101	94	74	13	-13
HCC-2998	97	100	104	48	-13
HCT-116	105	100	101	42	17
HCT-15	90	98	81	45	22
HT29	97	96	101	61	8
KM12	101	96	99	40	20
SW-620	100	93	92	58	36
CNS cancer					
SF-268	101	98	89	52	-46
SF-295	93	79	74	22	0
SF-539	83	90	87	1	-55
SNB-19	101	103	104	50	23
SNB-75	99	102	52	-11	16
U251	103	96	97	30	9
Melanoma					
LOX IMVI	91	91	85	45	25
MALME-3M	99	96	84	46	27
M14	90	98	98	43	5
SK-MEL-2	101	96	93	40	12
SK-MEL-28	106	90	76	46	43
SK-MEL-5	110	107	72	36	28
UACC-257	105	105	74	65	88
UACC-62	104	96	87	31	18
Ovarian cancer					
IGR-OV1	94	95	94	68	42
OVCAR-3	103	99	87	31	13
OVCAR-4	106	90	94	85	99
OVCAR-5	107	104	105	62	26
OVCAR-8	98	99	95	70	19
SK-OV-3	104	90	91	47	11
Renal cancer					
786-0	111	96	90	46	14
A498	104	97	1	11	1
ACHN	100	103	101	81	57
CAKI-1	95	72	46	36	33
RXF-393	90	93	8	52	41
SN12C	91	93	92	63	40
TK-10	92	93	97	91	71
UO-31	100	92	99	76	62
Prostate cancer					
PC-3	104	99	93	42	12
DU-145	100	97	94	17	-1
Breast cancer					
MCF7	104	100	94	48	44
MCF7/ADR-RES	102	98	97	61	23
MDA-MB-231/ATCC	97	67	72	64	22
HS 578T	103	92	87	51	53
MDA-MB-435	101	89	46	-25	-69
MDA-N	85	81	11	77	-83
BT-549	123	108	90	46	38
T-47D	99	100	86	59	77

Table 3a

Compound 1 ISO-Phomopsin A
ID No: 9409SC89

	Log10 Concentration Percent Growth				
Cell line	-6	-7	-6	-5	-4
Leukemia					
CCRF-CEM	103	97	92	7	-43
HL-60 (TB)	106	98	98	-29	-59
K-562	128	123	112	25	5
MOLT-4	97	105	106	46	5
RPMI-8226	106	104	87	0	-14
SR	96	99	94	28	5
Non-small cell lung cancer					
A549/ATCC	104	103	90	31	11
EKVX	103	101	98	64	58
HOP-62	95	82	79	53	21
NCI-H226	95	93	110	39	-15
NCI-H23	99	105	93	37	16
NCI-H322M	95	100	85	34	51
NCI-H460	95	96	85	7	-32
NCI-H422	100	99	95	10	-76
Colon cancer					
COLO 205	102	106	76	-45	-48
HCC-2998	96	99	92	15	-35
HCT-116	100	111	99	30	6
HCT-15	100	102	102	40	16
HT29	98	98	93	-30	-26
KM12	116	98	62	-33	-69
SW-620	99	99	87	23	2
CNS cancer					
SF-268	102	94	87	46	31
SF-295	100	93	88	-36	-52
SF-539	96	95	82	28	6
SNB-19	100	98	89	57	37
SNB-75	84	102	106	23	36
U251	97	93	83	15	-20
Melanoma					
LOX IMVI	99	96	91	43	19
M14	78	81	46	-6	-58
SK-MEL-2	100	95	80	18	0
SK-MEL-28	93	95	78	47	43
SK-MEL-5	117	110	41	2	1
UACC-257	98	96	85	20	27
Ovarian cancer					
IGROV1	105	106	94	49	37
OVCAR-5	102	100	95	30	25
OVCAR-8	103	107	105	66	32
SK-OV-3	105	106	86	24	35
Renal cancer					
786=0	93	94	99	40	24
A498	97	93	95	21	3
ACHN	101	95	91	46	21
SN12C	102	99	101	65	24
TK 10	101	103	101	87	58
Prostate cancer					
PC-3	101	100	85	26	15
DU-145	106	111	97	16	5
Breast cancer					
MCF7	102	92	69	20	3
MCF7/ADR-RES	106	109	95	19	2
MDA-MB-231/ATCC	102	100	107	64	28
HS 578T	103	80	69	38	31
MDA-MB-435	98	106	58	-26	-5
MDA-N	103	92	42	-7	-37
BT-549	116	108	103	71	32
T-47D	104	96	103	42	42

Table 3b

Compound 1 ISO-Phomopsin A
ID No 9502RM16

	Log10 Concentration Percent Growth				
Cell line	-6	-7	-6	-5	-4
Leukemia					
CCRF-CEM	103	106	97	25	-21
HL-60 (TB)	95	100	83	-19	-41
K-562	100	104	92	19	-17
MOLT-4	102	100	101	44	24
RPMI-8226	110	110	97	19	6
SR	100	96	41	-15	-22
Non-small cell lung cancer					
A549/ATCC	104	100	77	33	19
EKVX	94	101	96	44	22
HOP-62	94	94	89	54	26
HOP-92	96	96	76	66	86
NCI-H226	114	110	88	-6	-27
NCI-H23	104	105	83	48	39
NCI-H322M	106	98	96	32	14
NCI-H460	93	105	79	20	4
NCI-H522	101	97	88	15	-4
Colon cancer					
COLO205	90	76	44	-34	-70
HCC-2998	98	97	82	-8	-64
HCT-116	93	88	79	21	4
HCT-15	97	98	85	29	14
HT29	99	100	85	10	6
KM12	91	87	47	15	-6
SW-620	97	99	83	40	35
CNS cancer					
SF-268	94	92	88	52	37
SF-295	95	100	73	12	-11
SF-539	101	97	76	-29	-68
SNB-19	97	100	89	44	20
SNB-75	97	104	84	-3	60
U251	98	96	77	11	4
Melanoma					
LOX IMVI	98	100	92	45	30
MALME-3M	100	89	64	26	17
M14	101	80	69	5	-26
SK-MEL-2	94	99	75	23	17
SK-MEL-28	93	88	77	40	24
SK-MEL-5	99	87	60	29	35
UACC-257	84	92	78	46	58
UACC-62	96	96	83	45	29
Ovarian cancer					
IGR-OV1	98	97	93	58	36
OVCAR-3	97	90	46	3	-17
OVCAR-4	97	92	79	62	47
OVCAR-5	97	99	68	15	21
OVCAR-8	105	106	105	74	34
SK-OV-3	95	92	76	23	6
Renal cancer					
786-0	94	84	78	32	10
A498	82	84	87	[illegible]	[illegible]
ACHN	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CAKI-1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
RXF-393	100	102	75	42	35
SN12C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TK-10	101	105	97	96	71
U0-31	97	96	95	67	48
Prostate cancer					
PC-3	100	94	68	23	23
DU-145	107	100	72	15	9
Breast cancer					
MCF7	99	98	90	32	12
MCF7/ADR-RES	94	95	87	44	7
MDA-MB-231/ATCC	97	110	92	73	18
HS 578T	99	70	70	48	58
MDA-MB-435	103	87	34	-39	-69
DMDA-N	103	82	16	84	-68
BT-549	102	98	99	50	48
T-47D	84	96	71	41	5

Table 4a

Compound 1 Phomopsinamine A
ID No 9409SC89

	Log10 Concentration Percent Growth				
Cell line	-6	-7	-8	-5	-4
Leukemia					
CCRF-CEM	97	93	49	-13	-7
HL-60 (TB)	104	103	56	-53	-55
K-562	105	100	53	6	2
MOLT-4	95	93	91	26	17
RPMI-8226	106	103	65	9	
SR	104	95	71	26	12
Non-small cell lung cancer					
A549/ATCC	99	100	53	19	18
EKVX	89	96	80	44	38
HOP-62	105	101	75	29	34
NCI-H226	82	84	63	-12	-31
NCI-H23	110	112	78	11	-9
NCI-H322M	96	97	61	31	36
NCI-H460	104	111	37	4	-35
MNCI-H522	66	62	40	-42	-54
Colon cancer					
COLO 205	109	103	36	-22	-72
HCC-2998	109	113	60	-16	-59
HCT-116	99	99	63	14	1
HCT-15	93	94	76	19	2
HT29	101	102	50	-53	-64
KM12	110	123	77	47	40
SW-620	93	100	59	21	24
CNS cancer					
SF-268	101	100	70	32	13
SF-295	87	86	56	-22	-27
SF-539	97	102	77	-16	-44
SNB-19	86	94	65	27	20
SNB-75	63	78	36	11	22
U251	87	87	40	0	-39
Melanoma					
LOX IMVI	98	101	73	38	28
SK-MEL-2	102	96	37	-1	-6
SK-MEL-28	100	93	65	42	49
SK-MEL-5	102	99	32	17	14
UACC-257	95	95	66	31	42
Ovarian cancer					
IGROV1	100	98	69	37	19
OVCAR-5	102	93	60	11	15
OVCAR-8	81	103	85	55	2
SK-OV-3	101	107	59	27	14
Renal cancer					
786-0	100	98	91	31	15
A498	111	101	82	2	-11
ACHN	99	102	77	41	21
SN12C	96	93	82	33	13
TK-10	96	100	91	7	-1
UO-31	97	98	44	18	18
DU-145	99	99	48	17	1
Breast cancer					
MCF7	114	116	38	15	10
MCF7/ADR-RES	103	99	67	7	19
MDA-MB-231/ATCC	95	96	86	29	15
HS 578T	87	87	59	5	-5
MDA-MB-435	126	71	-15	-56	-32
MDA-N	93	87	-6	-58	-49
BT-549	145	85	51	29	-25
T-47D	99	105	71	29	68

Table 4b

Compound 1 Phomopsinamine A ID No 9502RM16	Log10 Concentration Percent Growth				
Cell line	-8	-7	-6	-5	-4
Leukemia					
CCRF-CEM	101	99	67	-7	-34
HL-60 (TB)	106	96	26	-37	-47
K-562	100	100	63	-3	-15
MOLT-4	104	99	88	25	9
RPMI-8226	107	99	61	6	4
SR	104	68	9	-15	-21
Non-small cell lung cancer					
A549/ATCC	97	81	44	21	7
EKVX		99	79	41	36
HOP-62	96	101	86	53	45
HOP-92	128	120	100	98	59
NCI-H226	114	104	47	-14	-27
NCI-H23	101	94	73	33	34
NCI-H322M	104	101	73	23	17
NCI-H460	100	102	87	12	14
NCI-H522	100	102	72	35	-24
Colon cancer					
COLO 205	98	82	28	-24	-45
HCC-2998		103	43	-32	-33
HCT-116	93	93	50	16	8
HCT-15		93	59	22	6
HT29	98	101	40	4	4
KM12	96	81	26	9	1
SW-620	97	93	71	40	31
CNS cancer					
SF-268	98	94	64	47	30
SF-295	103	81	24		0
SF-539	102	95	53	-45	-60
SNB-19	98	94	61	30	21
SNB-75	116	110	36	10	20
U251	104	89	41	11	12
Melanoma					
LOX IMVI		95	75	37	21
MALME-3M	100	78	55	29	25
M14	99	95	56	1	-1
SK-MEL-2	99	91	70	17	16
SK-MEL-28		86	60	24	45
SK-MEL-5	99	68	38	27	19
UACC-257	103	94	73	64	73
UACC-62	97	93	53	35	37
Ovarian cancer					
IGR-OV1	95	95	79	47	30
OVCAR-3	101	77	10	1	-6
OVCAR-4	98	102	89	59	45
OVCAR-5	99	105	50	25	35
OVCAR-8		102	92	42	22
SK-OV-3	100	97	51	0	4
Renal cancer					
786-0	105	101	53	25	20
A498		117	61	17	15
ACHN	102	101	71	53	36
UO-31	97	95	78	51	4
RXF-393	86	85	56	11	50
SN12C	96	97	79	45	31
TK-10		100	111	78	72
UO-31	102	104	97	53	53
Prostate cancer					
PC-3	102	86	38	20	19
DU-145	100	99	35	12	1
Breast cancer					
MCF7	103	106	43	27	9
MCF7/ADR-RES	97	96	85	46	21
MDA-MB-231/ATCC	96	90	76	27	2
HS 578T	102	72	70	62	77
MDA-MB-435		61	10	-42	-27
MDA-N	94	51	-58	-86	-65
BT-549	110	96	52	41	43
T-47D	93	100	77	54	78

***In vivo*, Hollow Fiber Screeening Assay**

The Biological Testing Branch of the Developmental Therapeutics Program has adopted a preliminary *in vivo* screening tool for assessing the potential anticancer activity of compounds identified by the large scale *in vitro* cell screen (Hollingshead, MG etal., Life Sciences, 57, 131 - 141, 1995). For these assays, human tumour cells are cultivated in polyvinylidene fluoride (PVDF) hollow fibers, and a sample of each cell line is implanted into each of two physiologic compartments (intraperitoneal and subcutaneous) in mice. The protocol identifies compounds having moderate to prominent anti-cancer activity, and facilitates identification of sensitive tumor lines and appropriate treatment regimens for subsequent testing in standard. *in vivo* solid tumor models.

Methodology

Each test mouse receives a total of 6 fibers (3 intraperitoneally and 3 subcutaneously) representing 3 distinct cancer cell lines. Three mice are treated with potential antitumor compounds at each of 2 test doses by the intraperitoneal route using a QD x 4 treatment schedule. Vehicle controls consist of 6 mice receiving the compound diluent only. The fiber cultures are collected on the day following the last day of treatment. To assess anticancer effects, viable cell mass is determined for each of the cell lines using a formazan dye (MTT) conversion assay. From this, the %T/C can be calculated using the average optical density of the compound-treated samples divided by the average optical density of the vehicle controls. In addition, the net increase in cell mass can be determined for each sample as a sample of fiber cultures are assessed for viable cell mass on the day of implantation into mice. Thus, the cytostatic and cytocidal capacities of the test compound can be assessed

Generally, each compound is tested against a minimum of 12 human cancer cell lines. This represents a total of 4 experiments since each experiment contains 3 cell lines. The data are reported as %T/C for each of the 2 compound doses against each of the cell lines with separate values calculated for the intraperitoneal and subcutaneous samples.

Evaluation

Compounds are selected for further *in vivo* testing in standard subcutaneous xenograft models on the basis of several hollow fiber assay criteria. These include: (1) a % T/C of 50 or less in 10 of the 48 possible test combinations (12 cell lines X 2 sites X 2 compound doses); (2) activity at a distance (intraperitoneal drug/subcutaneous culture) in a minimum of 4 of the 24 possible combinations; and/or (3) a net cell kill of 1 or more cell lines in either implant site. To simplify evaluation, a points system has been adopted which allows rapid viewing of the activity of a given compound. For this, a value of 2 is assigned for each compound dose which results in a 50% or greater reduction in viable cell mass. The intraperitoneal and subcutaneous samples are scored separately so that criteria (1) and (2) can be evaluated. Compounds with a combined IP+SC score $\geq$ 20, a SC score $\geq$ 8 or a net cell kill of one or more cell lines are referred for xenograft testing. These criteria were statistically validated by comparing the activity outcomes of > 80 randomly selected compounds in the hollow fiber assay and in the xenograft testing. This comparison indicated that there was a very low probability of missing an active compound if the hollow fiber assay were used as the initial *in vivo* screening tool. In addition to these criteria, other factors (e.g. unique structure, mechanism of action) may result in referral of a compound for standard xenograft testing without the compound meeting these criteria.

Results

The data acquired for phomopsin A demonstrated significant cell growth inhibition and cytocidal activity as demonstrated by the %T/C results shown for various cell lines in Table 5

Table 5

Hollow fibre assay (%test/control, %T/C) for Phomopsin A

Cell line	30mg/kg/dose IP	30mg/kg/dose SC	20mg/kg/dose IP	20mg/kg/dose SC	45mg/kg/dose IP	45mg/kg/dose SC	30mg/kg/dose IP	30mg/kg/dose SC
Expt591					**Expt580**			
LOX IMVI	>100 ; >100	>100 ; >100	37 , 29	>100 , >100	98 ; 98	80 ; 84	88 , 90	85 ; 88
COLO 205	67 ; 59	49 ; 34	58 ; 48	85 , 81	>100 ; >100	64 ; 72	58 ; 67	86 ; 89
OVCAR-3	35 ; -18	79 ; 22	36 ; -15	>100 ; >100	61 ; 79	>100 ; >100	25 ; 60	37 ; 66
Expt590					**Expt579**			
NCI-H23	81 ; 76	96 ; 94	>100 ; >100	>100 ; >100	44 ; 68	-41 ; 38	60 ; 77	21 ; 65
MDA-MB-231	46 ; 30	72 ; 63	44 ; 28	46 ; 29	>100 ; >100	>100 ; >100	99 ; 99	>100 ; >100
SW-620	>100 ; >100	>100 ; >100	>100 ; >100	>100 ; >100	78 ; 82	78 ; 83	97 ; 98	86 ; 89
Expt581								
NCI-H522	85 ; 59	96 ; 91	70 ; 18	>100 ; >100				
UACC-62	100 ; 100	92 ; 81	97 ; 95	90 ; 79				
U251	>100 , >100	95 ; 91	90 , 83	99 ; 98				
Expt582								
MDA-MB-435	71 ; 62	69 ; 58	81 ; 74	87 , 82				
OVCAR-5	51 , 38	92 , 90	89 , 87	95 ; 94				
SF-295	89 , 68	>100 , >100	>100 , >100	>100 , >100				

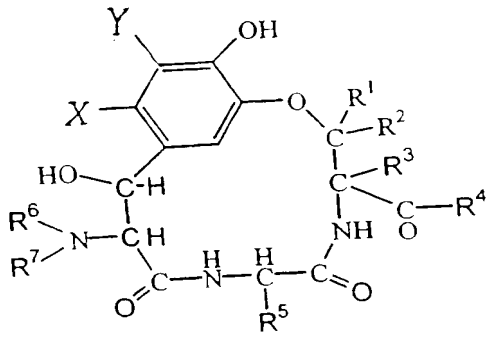
Data are results from duplicate assessments against implanted cell lines
IP = intraperitoneal
SC = subcutaneous

Finally, it is to be understood that various alterations, modifications and/or additions may be introduced into the composition and/or arrangement of steps previously described without departing from the spirit or ambit of the invention.

Claims

1. A method of treatment of a patient suffering cancer comprising administering to the patient an effective amount of a phomopsin.

2. A method according to claim 1 wherein the patient is treated with a compound selected from compounds of formula I and derivates thereof



(I)

wherein:

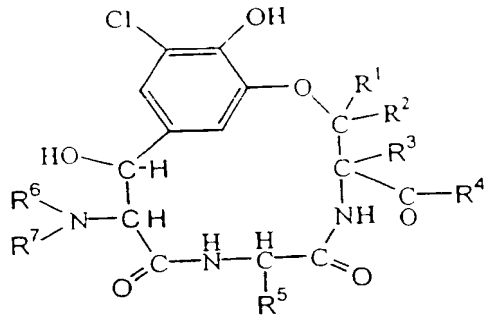
R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are optional substituents

X is selected from the group consisting of aliphatic, hydrogen and halogen; and

Y is selected from the group consisting of aliphatic, hydrogen and halogen.

3. A method according to claim 2 wherein the patient is treated with a compound selected from compounds of formula I and derivatives and salts thereof wherein in said compound of formula I the substituent X is hydrogen, Y is chlorine and R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are independently selected from the group consisting of hydrogen aliphatic aromatic peptide chains and halogen and wherein a conjugate may be formed between a peptide chain and a monoclonal antibody.

4. A method according to claim 1 wherein the patient is treated with an effective amount of a compound of formula Ia or derivative or salt thereof



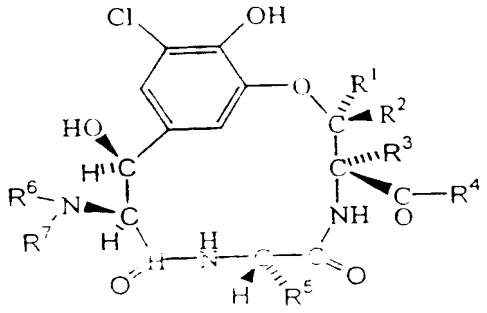
(Ia)

wherein R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are independently selected from hydrogen and aliphatic and R^4 is a peptide optionally conjugated with an antibody.

5. A method according to claim 4 wherein R^1, R^2, R^5 and R^6 are lower aliphatic and R^3 and R^7 are hydrogen.

6. A method according to claim 4 wherein R^1 is ethyl, R^2 is methyl, R^3 is hydrogen, R^5 is isopropyl or iso-propenyl and R^6 is methyl and R^7 is hydrogen.

7. A method according to any one of claims 4 to 6 wherein the phomopsin of formula Ia comprises compounds of the stereochemistry Ib

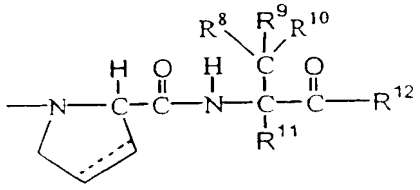


(Ib)

8 A method according to claim 7 wherein at least 60% by weight of phomopsins present are stereochemistry Ib.

9. A method according to any one of claims 2 to 7 wherein R^4 is a di or tripeptide optionally bound to an antibody.

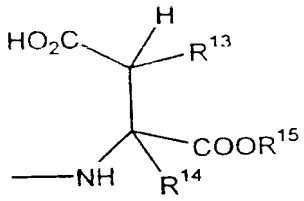
10. A method according to claim 8 wherein R^4 has the formula II with all possible stereochemical permutations



(II)

wherein the dotted lines represents an optional double bond; R^8 and R^9 are independently selected from hydrogen and lower alkyl; and R^{10} and R^{11} are hydrogen, or together make a double bond andR^{12} is selected from the group consisting of amino, mono substituted amino, disubstituted amino and an amino acid residue.

11. A method according to claim 10 wherein R^{12} is of formula III



(III)

wherein R^{13} and R^{14} are hydrogen or together form a double bond and R^{15} is selected from the group consisting of hydroxy, amino, substituted amino and a monoclonal antibody.

12. A method according to claim 1 wherein the patient is treated with a phomopsin selected from the group consisting of phomopsin A, octahydrophomopsin A, iso-phomopsin A, phomopsinamine A, salts thereof and mixtures of two or more thereof

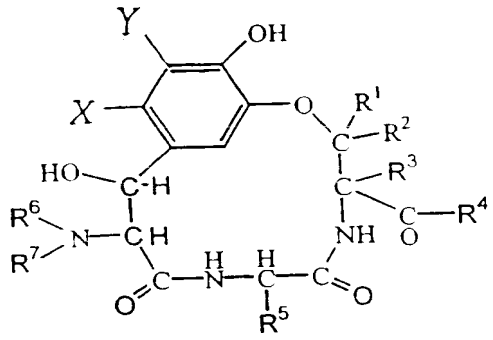
13. A method according to any one of claims 1 to 12 wherein the patient is suffering from liver cancer.

14. A method according to any one of claims 1 to 13 wherein said phomopsin or derivative thereof is administered in a pharmaceutical composition with a pharmaceutically acceptable carrier.

15. A method according to any one of claims 1 to 14 wherein the patient is also treated with one or more other anticancer drugs in combination with phomopsin.

16. A method according to any one of claims 1 to 15 wherein the administration of phomopsin is at a dosage to effect anticancer activity without adverse cytotoxic effects on normal cells.

17. A pharmaceutical composition for treatment of cancer comprising a compound of formula I or derivative thereof and a pharmaceutically acceptable carrier therefore



(I)

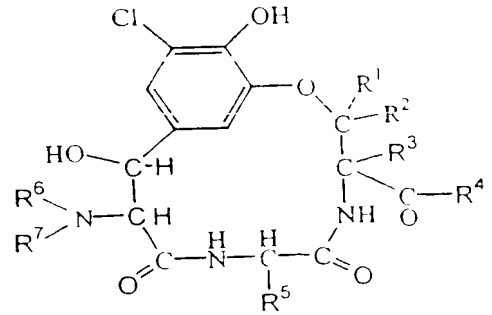
wherein

R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are optional substituents

X is selected from the group consisting of aliphatic, hydrogen and halogen; and

Y is selected from the group consisting of aliphatic, hydrogen and halogen.

18 A pharmaceutical composition for treatment of cancer comprising a compound of formula Ia or salt thereof



(Ia)

wherein R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are independently selected from hydrogen and aliphatic and R^4 is a peptide optionally conjugated with an antibody.

19. A pharmaceutical composition according to claim 17 wherein the phomopsin or derivative thereof is selected from the group consisting of phomopsin A, octahydrophomopsin A, isophomopsin A, phomopsinamine A, salts thereof and mixtures of two or more thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01193

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. A61K 38/12, A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 38/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, CAPLUS, MEDLINE; keywords, phomopsin, cancer, tumor.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN ASWEGEN, C. H. et al "INFLUENCE OF PHOMOPSIN AND IVALIN ON STEROID-HORMONE BINDING AND GROWTH OF MCF-7 HUMAN BREAST CANCER CELLS." Journal of Toxicology and Environmental Health, VOL 16(1), 1985 pages 13-23. See whole document	1-19
X	LI, YIN et al "INTERACTION OF PHOMOPSIN A WITH PORCINE BRAIN TUBULIN." Biochemical Pharmacology, Vol 43, No 2, pages 219-224, 1992. See whole document.	17-19

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:
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Date of the actual completion of the international search
19 October 2000

Date of mailing of the international search report
1 NOV 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01193

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKAHASHI, M et al "SYNTHETIC STUDY OF USTILOXIN ANALOGS: BENZYLIC OXIDATION OF 13-MEMBERED CYCLIC PEPTIDE BY LEAD TETRAACETATE." HETEROCYCLES, Vol. 47, No 1, 1998 pages 163-166. See Whole document.	17-19
X	AU 64916/90 (643464)B (CSIRO) 23 October 1990. See Whole document.	17-19